

An evaluation of methods to determine the porosity of calcium phosphate cements

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Abstract: The porosity of a material can be determined using a diversity of methods; however, the results from these methods have so far not been compared and analyzed for calcium phosphate cements (CPCs). The aim of this study was to compare a fast and easy method for porosity measurements with some commonly used porosity methods for CPCs. The investigated method is based on the assumption that when a wet cement sample is dried, the volume of the evaporated water is equal to the volume of pores within the cement. Moreover, different methods of drying the cements were evaluated for acidic CPCs. The results showed that drying at room temperature ($22 \pm 1^\circ\text{C}$) is preferable, since a

phase transformation was observed at higher temperatures. The results also showed that drying for 24 h in vacuum was sufficient to achieve water-free cements. The porosity measured was found to vary between the porosity methods evaluated herein, and to get a complete picture of a cement's porosity more than one method is recommended. Water evaporation, is, however, a fast and easy method to estimate the porosity of CPCs and could simplify porosity measurements in the future. © 2014 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 00B:000–000, 2014.

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INTRODUCTION

Calcium phosphate cements (CPCs) have been studied since the early 1980s,^{1,2} and there is still plenty of ongoing research within this area. Since these cements are very similar to the mineral phase of bone they have a high biocompatibility. However, their main limitation in comparison to the more traditionally used acrylic cements are their mechanical properties, leading to numerous studies focusing on improving the mechanical resistance of CPCs. Since the mechanical properties are strongly linked to the porosity (and the pore size distribution) of ceramic materials,^{3,4} it is of high interest to have reliable and fast methods to determine the porosity of such materials.

Depending on the starting powders used, the CPCs can have either a basic or acidic setting reaction; the basic cements having precipitated hydroxyapatite (PHA) as the end product; the acidic cements having dicalcium phosphate dihydrate (brushite) and/or dicalcium phosphate anhydrous (monetite) as their end products. Studies on methods to determine porosity⁵ and obtain dry materials⁶ have been performed for cements used in the field of construction and has furthermore been thoroughly reviewed in "Water Transport in Brick, Stone and Concrete" by Hall and Hoff.⁷ These studies showed that the determined porosity is highly affected by the drying procedure and porosity method used. However, to the authors' knowledge, no such evaluation has

been made for CPCs. Compared to cements used for, for example, construction, the CPCs have different setting reactions, final phase composition, and setting times. There are therefore reasons to believe that the CPCs do not behave in the same way during porosity measurement and drying as the construction cements. It is hence important to evaluate the drying procedures and compare porosity measurements for CPCs when utilizing different porosity methods in order to determine which methods can be used and which should be avoided.

Porosity can be measured using a diversity of methods and depending on the material analyzed different methods are more commonly used. For CPCs, mainly three methods are utilized: helium pycnometry,^{8–10} mercury intrusion porosimetry (MIP),^{3,11,12} and using the tabulated density value of the material^{11,13–15} to calculate the porosity. The main drawbacks of these methods are the destructiveness, the long analysis times and the need for special equipment, especially for MIP and helium pycnometry, which may entail that fewer samples are analyzed, compromising statistical power. Mercury is additionally expensive and toxic; requiring careful handling, hence adding complexity to the method. The presence of amorphous phases and unreacted starting materials could furthermore give misleading results when using the tabulated density (presupposes a 100 pure and crystalline material) for the calculations.

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In this study, an alternative method to measure the porosity of CPCs is compared to some common methods used for CPCs and other types of cements. The new, alternative method utilizes the assumption that the volume of evaporable water in a wet sample is equal to the volume of the voids within the sample, the accuracy of which has not been discussed before for CPCs. However, the method is commonly being used for other cements such as Portland cement and concrete.^{5,6} This method is the reverse of the water resaturation method, which is also frequently used for other types of cements,^{5,7} and the only equipment needed is a balance and a method to measure the sample volume (using, e.g., a caliper, or Archimedes method in water or mercury), which makes it a fast and easy method for porosity measurements.

The aims of this study were to evaluate a few of the existing porosity methods and to develop a method for porosity measurements of CPCs that is fast, easy and cheap; that is, do not require any advanced equipment. The method should also include a drying procedure that does not result in a compositional change for the investigated CPCs.

THEORY

When discussing porosity, different expressions are used for the density; and a clarification of the terminology follows. Throughout this article, *apparent density* describes the density of a sample when the outer dimensions are used to measure its volume. *Skeletal density* describes the density achieved when calculating the volume of the solid material, excluding pores.

Two of the commonly used methods (i.e., tabulated density and helium pycnometry) calculate the porosity of the material from the division of the apparent density with the skeletal density according to Eq. (1), where ρ_a is the apparent density, and ρ_s is the skeletal density. The apparent density is normally measured by immersion in mercury using Archimedes principle,¹¹ or by measuring the outer diameters of the sample.³

$$\Phi(\%) = \left(1 - \frac{\rho_a}{\rho_s}\right) \cdot 100 \quad (1)$$

For basic CPCs it is common to use the tabulated density of the material [i.e., 3.16 g/cm³ for hydroxyapatite (HA)] as the skeletal density in Eq. (1).^{11,13-15} However, for the acidic CPCs helium pycnometry is more commonly used.⁸⁻¹⁰ Helium pycnometry measures the skeletal volume of the samples by determining the difference in helium pressure between an empty chamber with a calibrated volume and the chamber containing a sample.¹⁶

The last, and most advanced, method used on CPCs is MIP, which measures pressure differences during intrusion of mercury into the pores.¹⁷ From this method not only the total porosity can be achieved, but also the size distribution of the pore interconnections. The CPCs that have been tested with this method have so far mostly been basic CPCs.^{3,11,12}

Solvent resaturation is a method that is commonly used for other types of porous materials.^{5,7} In this method, dry

samples are immersed in a solvent, normally water or alcohol and the weight change is monitored until a constant weight is achieved. The porosity is then calculated according to Eq. (2), where m_{sat} is the weight of the sample saturated with solvent, m_d is the dry weight, ρ_{solvent} is the density of the solvent used, and V_a is the apparent volume.

$$\Phi(\%) = \left(\frac{(m_{\text{sat}} - m_d)/\rho_{\text{solvent}}}{V_a}\right) \cdot 100 \quad (2)$$

The next method used on other materials is solvent exchange, where wet samples are stored in a new solvent (normally an alcohol) and the weight reduction is monitored during the exchange process.¹⁸ When a constant weight is achieved, the porosity is calculated according to Eq. (3), where m_{water} is the weight of wet sample, m_{alcohol} is the weight after complete exchange, ρ_{water} is the density of water, and ρ_{alcohol} is the density of the alcohol used.

$$\Phi(\%) = \left(\frac{(m_{\text{water}} - m_{\text{alcohol}})/(\rho_{\text{water}} - \rho_{\text{alcohol}})}{V_a}\right) \cdot 100 \quad (3)$$

All above discussed methods have the disadvantage of not being able to reach all pores. Depending on the radius of the molecule used, different pore sizes can be reached; however, some pores will always be considered as closed pores and will not be taken into account in the calculations. The density of the actual bulk material can in theory be calculated using molecular weight and crystalline lattice parameters received from X-ray diffraction (XRD) measurements.¹⁶ However, XRD calculations do not take into account any amorphous phases that can be present in the sample and might therefore overestimate the skeletal density.

MATERIALS AND METHODS

Acidic CPC sample preparation

Equimolar amounts of monocalcium phosphate monohydrate (Scharlau) and beta-tricalcium phosphate (β -TCP, Sigma-Aldrich) were thoroughly mixed with 1 wt % disodium dihydrogen pyrophosphate (SPP, Sigma-Aldrich). The powders were blended with 0.5 M citric acid (aq) in a liquid to powder ratio (L/P) of 0.3 mL/g, this L/P was chosen since it produces a very liquid paste with a long setting time (i.e., 35 min initial setting time with the Gillmore needle method¹⁹), from which air easily can escape. Defects from mixing will hence be minimized. Both SPP and citric acid were added to retard the setting reaction.

The cement paste was transferred to cylindrical rubber molds (\emptyset 6 mm, height 12 mm), and allowed to set for 40 min at room temperature [RT, 22°C (\pm 1°C)]. The cement samples were then immersed in 40 mL of phosphate buffered saline (PBS) solution (Sigma-Aldrich, 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, pH 7.4) in a sealed beaker and stored at 37°C for 24 h. The sample volume was measured using Archimedes principle in double distilled water at RT. To ensure that all pores were filled with water/PBS at the

TABLE I. Mass Absorption Coefficients (μ/ρ) for the Different Calcium Phosphates, Calculated With Eq. (7) and Tabulated Values²⁵

Component	μ/ρ
β -TCP	86.5
β -CPP	77.7
Brushite	60.3
Monetite	73.3

point when the apparent volume was measured, some samples were put in PBS in a vacuum chamber (250 mbar) for 2 min to extract all air from the samples, and then weighed before and after this treatment, at an accuracy of 0.01 g. Since no weight difference could be seen it was concluded that CPC samples stored in PBS for 24 h can be considered completely wet.

Evaluation of drying methods

Different drying methods were evaluated in order to find one or more methods that were fast and did not affect the sample composition during drying. The acidic CPC samples were dried using four different methods; under vacuum (250 mbar) at RT (1), in a desiccator with a silica gel drying agent at RT (2), 37°C (3), and 60°C (4). The samples were weighed after 6 h, and 1, 2, 3, 5, and 7 days, except for the samples dried at 37°C, which were further weighed until they were considered dry. The samples were considered dry when the weight loss rate was drastically reduced, corresponding to a weight change of less than 0.5 wt % per day.

To verify that no reaction occurred during drying, the cement phases before and after drying were analyzed using XRD (D8, Bruker) in a theta–theta setup with Cu-K α irradiation. Diffraction angles (2θ) 10–60° were analyzed in steps of 0.034° with 0.75 s per step, a sample rotation speed of 80 rpm, and using a nickel filter. The samples were carefully ground before analysis, and three measurements were made for each group. The composition was calculated using Rietveld refinement with BGMN software (BGMN, Germany); with the reported result being the mean of three measurements with the relative error as $2.77 \times$ standard deviation according to ASTM E177-13.²⁰ The structures used for the refinement were; monetite from PDF #04-009-3755,²¹ brushite from PDF #04-013-3344,²² β -TCP from PDF #04-008-8714,²³ and β -calcium pyrophosphate (β -CPP) from PDF #04-009-3876.²⁴ Mass absorption coefficients for the phases analyzed with XRD can be found in Table I, and are calculated by Eq. (4),²⁵ where μ/ρ is the mass absorption coefficient for the compound, w_n is the weight fraction of element n , and $(\mu/\rho)_n$ is the mass absorption coefficient for element n .

$$\frac{\mu}{\rho} = w_1 \left(\frac{\mu}{\rho} \right)_1 + w_2 \left(\frac{\mu}{\rho} \right)_2 + \dots + w_n \left(\frac{\mu}{\rho} \right)_n \quad (4)$$

Porosity measurements

In total, six different methods of measuring the porosity were used on the acidic CPCs: the fast, alternative method

of water evaporation (1) was compared to three of the most commonly used methods for CPCs—helium pycnometry (2), calculations based on XRD measurements (3), and MIP (4)—as well as two other methods commonly used for other cements—methanol resaturation (5) and methanol exchange (6). All drying methods were evaluated for porosity methods 1, 2, 3, and 5, while only the one giving the best results was used for MIP, due to the low cost-effectiveness of this method (no drying was needed for porosity method 6).

The apparent volume (V_a) of all samples was measured as previously described in water, utilizing a balance (ML 104, Mettler Toledo AB, 0.0001 g, Switzerland) with a density kit (ML-DNY 43, Mettler-Toledo AB, Switzerland), for the measurements. The apparent density (ρ_a) was then calculated from the weight of the samples after drying (m_d), according to the following equation:

$$\rho_a = \frac{m_d}{V_a} \quad (5)$$

Water evaporation. The wet weight of the sample was measured right after removal from the PBS. PBS that had been adsorbed on the surface was removed using moist Kimwipes[®] tissue paper before weighing. The difference in weight before and after drying was used to calculate the water content in the samples, with 20 samples per group. The porosity was calculated according to Eq. (6), where V_w is the volume of the evaporated water, and V_a is the apparent volume of the samples (measured as detailed in the previous paragraph):

$$\Phi(\%) = \left(\frac{V_w}{V_a} \right) \cdot 100 \quad (6)$$

Helium pycnometry. The skeletal densities of the samples were determined with a helium pycnometer (AccuPyc 1340, Micromeritics, UK, maximum pressure of 19.5 Psi, chamber size of 1 cm³) using 20 purges and 10 runs. Before measurement, 10 samples from each group were ground and homogenized. Out of the ground entity, three equal powder samples were taken and further analyzed. The porosity was calculated using the skeletal density and the apparent density according to Eq. (1).

Calculated density from XRD. The skeletal density was calculated using the phase compositions resulting from the XRD measurements. Densities that were used in the calculations were calcium pyrophosphate 3.09 g/cm³, TCP 3.14 g/cm³, brushite 2.33 g/cm³, and monetite 2.93 g/cm³. The porosity was calculated using the calculated skeletal density and the apparent density according to Eq. (1).

Methanol resaturation. After the samples had been dried according to the previously described methods, 10 samples were immersed in 3 mL of methanol, and the weight change

was monitored regularly. When no weight change was noted (0.01 g accuracy), saturation was assumed to have occurred and the porosity was calculated using Eq. (2), where 0.792 g/cm³ was used as the density of methanol.

Mercury intrusion porosimetry. To further compare water evaporation with commonly used porosity methods, cement samples were analyzed with MIP (AutoPore III, Micromeritics). The porosity was calculated from the amount of volume that could be intruded by mercury when a 130° contact angle and a surface tension of 485 mN/m were assumed. Three samples that had been dried in vacuum for 24 h were tested with this method (drying method 1).

Methanol exchange. Wet samples (10) were immersed in 10 mL of methanol (approximately 100 times the volume of water within the pores) at RT, and the weight change was monitored regularly. Methanol was changed after 6 and 24 h to ensure a complete exchange. The exchange was deemed completed when no weight change (0.01 g accuracy) could be monitored between two time points. The porosity was calculated according to Eq. (3), where 0.792 g/cm³ was used as the density of methanol.

Statistical analysis. IBM SPSS Statistics v.21 was used to perform the statistical analyses. A one-way analysis of variance (ANOVA) was used to evaluate the weight change during drying for each drying method. Scheffe's *post hoc* test was used to evaluate differences between time points since equal variances could not be confirmed for all groups. A general linear model (GLM) analysis was performed to evaluate the effect of porosity method and drying method on the cement porosity, and parameter estimates were calculated for all direct and interactive factors. A significance level of $\alpha = 0.05$ was used for all tests.

Validation of method with HA cement (Calcibon®)

The accuracy of the alternative porosity method was further validated using a basic CPC (Calcibon®) and the drying method that gave the best results for the acidic CPC, that is, drying in vacuum at RT for 24 h. Calcibon was mixed in an L/P according to the manufacturer's instructions for use. The cement was molded similar to the acidic CPC and after 10 min of curing the cement was transferred to 40 mL of PBS and stored at 37°C for 24 h. Calcibon was only evaluated using the most commonly used methods for CPCs, that is, helium pycnometry and tabulated density/XRD, together with the water evaporation method. Samples for porosity measurements were treated similar to the acidic CPC samples. For Rietveld analysis from XRD measurements, the additional PDF files used was taken from PDF #01-074-0565 (HA),²⁶ PDF #04-010-4348 (α -TCP),²⁷ and PDF #04-008-0788 (calcite).²⁸ Furthermore, the densities used for theoretical density calculations were, in addition to the ones used for the acidic CPCs also, HA 3.16 g/cm³, and calcite 2.71 g/cm³. For Calcibon, the porosity was also calculated using the theoretical density of pure HA as the bulk density, as in previous publications within the area.¹³⁻¹⁵ A one-way

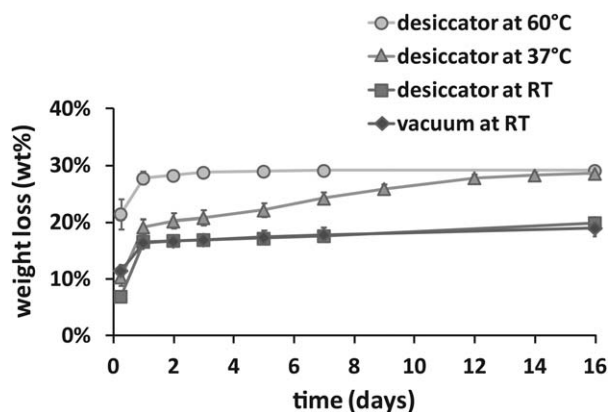


FIGURE 1. Total weight loss for the acidic CPCs at different time points for the four different drying methods. Error bars indicate standard deviations for $n = 8$.

ANOVA was performed on the porosity results at a significance level of 0.05. Since Levene's test did not confirm homogeneity of variances, Tamhane's *post hoc* test was used to evaluate differences between groups.

RESULTS

Evaluation of drying methods

The total weight change for the acidic CPCs as a function of time can be seen in Figure 1. Although a small weight change for all samples dried at RT was seen after 24 h, this change is likely due to a continuous phase transformation from brushite to monetite, which is not desired, and samples dried in both desiccator and vacuum at RT were hence deemed dry after 24 h. The weight change corresponding to dry samples was around 17 wt %. Furthermore, a faster initial weight loss was seen when drying in vacuum than in desiccator, when the tests were performed at RT. At the time the samples dried at higher temperatures were deemed dry a weight change of approximately 28–29 wt % had occurred. This happened after 24 h at 60°C and after about 2 weeks at 37°C, and although small changes were

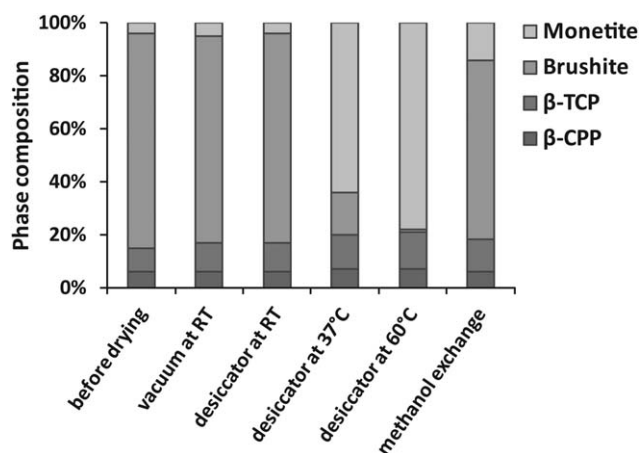


FIGURE 2. Phase composition for the different treatments of the set cement. Results are from XRD and Rietveld refinement. Relative errors for $n = 3$ was always equal to or lower than 1.5 wt %.

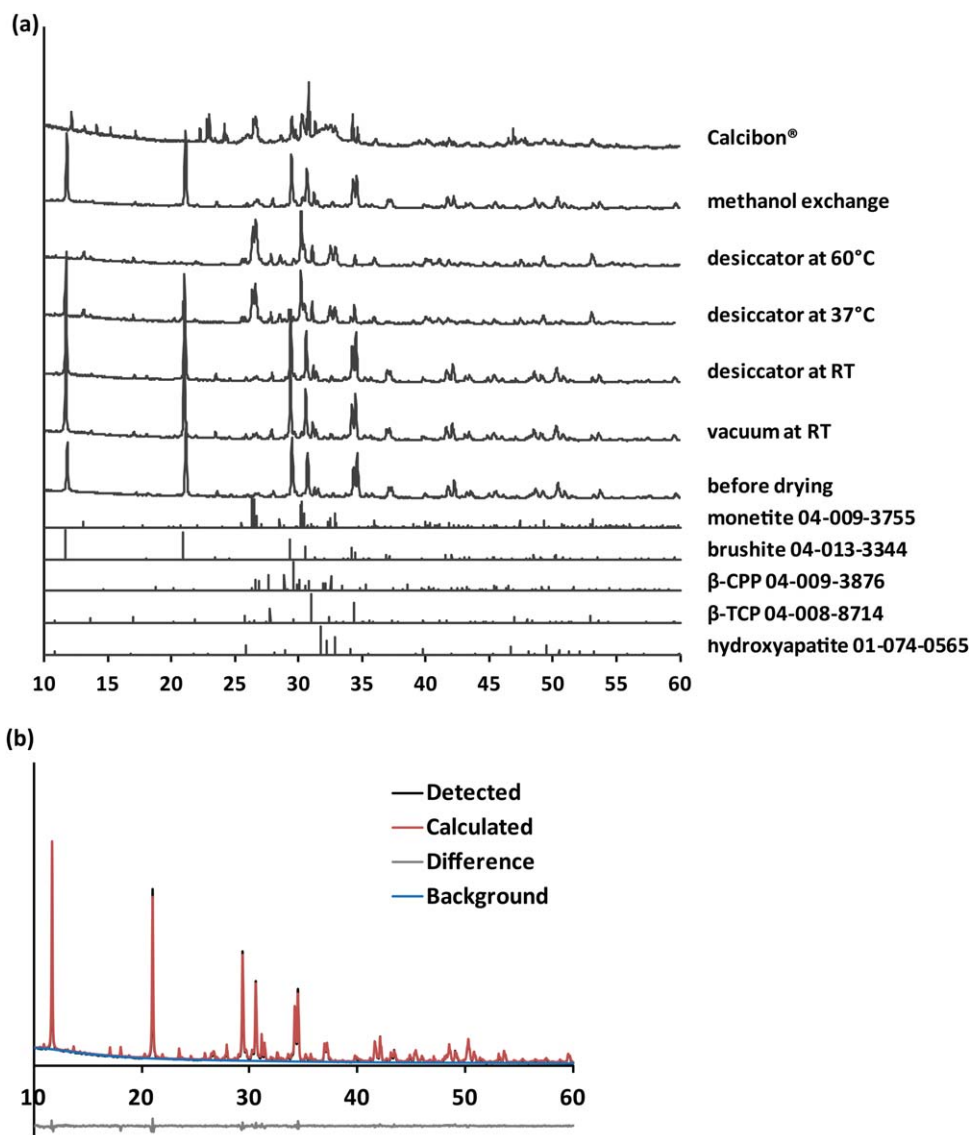


FIGURE 3. (a) Representative XRD plots for all compositions (one of three measurements is shown per composition). (b) Example of Rietveld refinement accuracy as calculated by BGMN software (acidic CPC dried in vacuum at RT for 24 h). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

seen even after these time points, the differences were not statistically significant ($p > 0.05$).

Results from the XRD analysis showed that the same amount of monetite (around 5 wt %) could be found in the samples before and after drying for 24 h in both desiccator and vacuum at RT, see Figure 2. However, a small decrease in brushite content, and an associated small increase in β -TCP content, was seen after drying at all temperatures. Furthermore, the samples dried at higher temperatures showed a substantial increase in monetite content, with a close to complete conversion from brushite to monetite for the samples dried at 60°C, and a decrease from around 82 wt % brushite before drying to around 16 wt % after drying at 37°C, see Figure 2. XRD results for the samples that had been through the methanol exchange process showed an increase in monetite content from around 5 wt % before

drying to almost 14 wt % after drying, see Figure 2. No change in β -CPP was seen between any samples. XRD patterns and a representative example of the accuracy of the Rietveld refinement are shown in Figure 3.

Porosity measurements

The skeletal density calculated from XRD and Rietveld refinement of the wet samples was 2.46 g/cm^3 ($\pm < 0.01 \text{ g/cm}^3$), which is similar to the calculated density after drying at RT, both in vacuum and in desiccator, see Table II. However, samples dried at higher temperatures showed higher skeletal density. The density calculated from XRD was always slightly higher than the skeletal density measured with helium pycnometry, see Table II. Results also showed that the apparent density was lowest for the samples that had been dried at the highest temperature.

TABLE II. Density Measured/Calculated for the Different Methods, Standard Deviation Within Brackets

Sample	Helium Pycnometry (g/cm ³)	XRD Calculations (g/cm ³)	Apparent Density (g/cm ³)
Dried in vacuum at RT (24 h)	2.41 (0.01)	2.50 (<0.01)	1.58 (0.03)
Dried in desiccator at RT (24 h)	2.40 (<0.01)	2.49 (<0.01)	1.62 (0.04)
Dried in desiccator at 37°C (2 weeks)	2.81 (0.01)	2.87 (<0.01)	1.39 (0.04)
Dried in desiccator at 60°C (24 h)	2.83 (<0.01)	2.97 (<0.01)	1.32 (0.02)
Wet sample	–	2.46 (<0.01)	–

Samples dried in vacuum at RT showed the smallest difference within the group; only 3% difference in porosity between the highest (XRD) and the lowest (water evaporation) method, see Figure 4. XRD calculations tended to give higher porosity values than helium pycnometry, which in turn tended to give higher values than methanol resaturation, although these differences were not statistically significant, see Table III. However, the relative results of the water evaporation method differed between drying methods. It gave significantly lower porosity than the helium pycnometry and XRD calculations for drying at RT, but somewhat higher porosity than the other methods when drying at the higher temperatures, see Table III. The average porosity for each method of drying was: 33.4% (vacuum at RT), 30.8% (desiccator at RT), 49.7% (desiccator at 37°C), and 53.9% (desiccator at 60°C). Drying in a desiccator at RT gave significantly lower porosities than all other drying methods, vacuum drying gave significantly lower values than drying

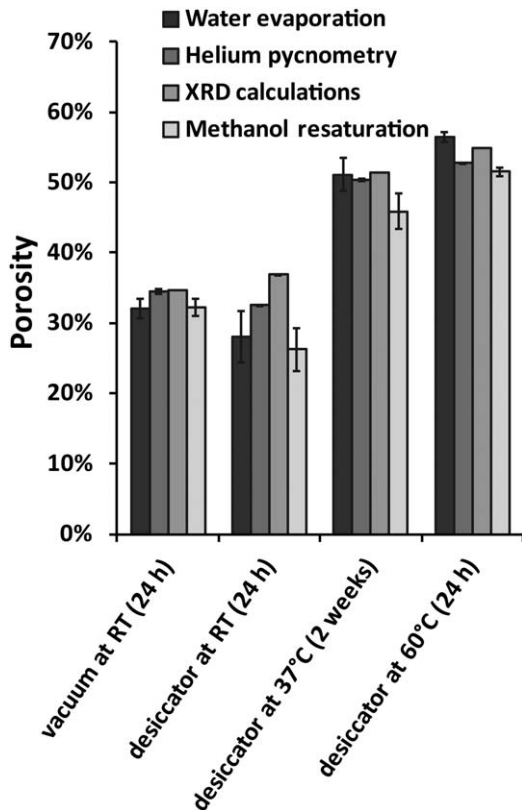


FIGURE 4. Porosity of the samples for different ways of drying and analysis techniques.

at 37 and 60°C, and no significant difference could be seen between drying at 37 and 60°C, see Table III.

Mercury intrusion porosimetry. The total porosity measured with MIP on the samples dried in vacuum at RT was 29.0% (1.3%), which is slightly lower than the results achieved from the other methods. The size distribution showed that most pores were around 1 μm in size, but also some smaller and larger could be noted, see Figure 5. Furthermore, no pores larger than approximately 30 μm were seen.

Methanol exchange. The porosity method that did not include any drying, that is, methanol exchange, showed a porosity of 41.0% (2.6%), which is in between the values achieved when drying at RT and at higher temperatures.

Validation of method with HA cement (Calcibon®)

The XRD measurements on Calcibon showed a slight compositional change during drying; a decrease of about 5 wt % in HA and a corresponding increase in α-TCP was seen after drying for 24 h in vacuum, see Table IV. The remainder of the phases had a similar composition before and after drying. Since a change from HA to α-TCP releases some water this could have an effect on the measured water evaporation, and in turn also on the final porosity, albeit not so large. Since the density of HA and α-TCP are quite similar (3.16 and 3.14 g/cm³, respectively) the density before and after drying was also similar, close to 3.07 g/cm³. However, a large difference could be seen between this calculated density and the density measured with helium pycnometry,

TABLE III. Results From the GLM Analysis

Parameter	Coefficient (%)	Significance (p)
Intercept	52.8	<0.001
Drying in vacuum at RT	-18.3	<0.001
Drying in desiccator at RT	-20.3	<0.001
Water evaporation	3.7	0.005
Drying in vacuum at RT × water evaporation	-6.1	0.001
Drying in desiccator at RT × water evaporation	-8.1	0.001
Drying in desiccator at RT × methanol resaturation	-5.0	0.011

Only significant factors and interactions are shown (p < 0.05). Drying at 60°C and helium pycnometry corresponds to the intercept.

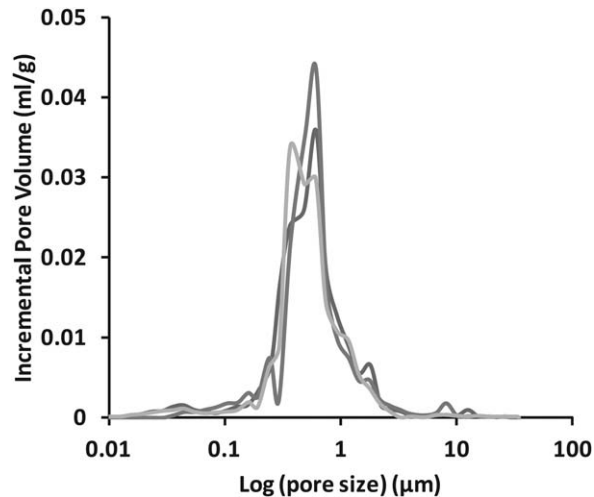


FIGURE 5. Size distribution of the three acidic CPC samples dried in vacuum for 24 h at RT analyzed with MIP.

which was as low as 2.63 g/cm³. A representative XRD pattern for Calcibon can be seen in Figure 3(a).

The porosity measured for Calcibon showed larger differences than for the brushite cement and all methods (water evaporation, He pycnometry, XRD, and tabulated HA) were significantly different to each other ($p < 0.001$). Both water evaporation and helium pycnometry gave values around 35%, while using the theoretical density from XRD data gave values of around 10% higher, see Table V. Furthermore, using the tabulated density of pure HA gave even higher values, although still comparable with the theoretical density from XRD measurements.

DISCUSSION

The results presented in this study show that water evaporation can indeed be used as a reliable estimation of the porosity of CPCs. The method is fast, cheap, easy, and can be used for all types of CPCs. The method must, however, be performed according to the steps below to ensure the accuracy shown here.

1. Cure the prepared samples in PBS or water for at least 24 h to guarantee fully wetted samples.
2. Weigh the samples and measure the apparent volume (using, e.g., a caliper or Archimedes method in water or mercury) before drying.
3. Dry samples in vacuum (250 mbar or lower) at RT ($22 \pm 1^\circ\text{C}$) for 24 h.

TABLE IV. Composition Before and After Drying Calculated for Calcibon From XRD Measurements, Standard Deviation Within Brackets

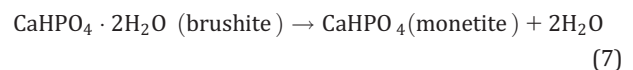
	α -TCP (wt %)	β -TCP (wt %)	HA (wt %)	Monetite (wt %)	Calcite (wt %)
Wet	24 (1)	2 (3)	46 (6)	23 (2)	6 (1)
Dry	28 (1)	2 (1)	41 (2)	23 (1)	6 (1)

TABLE V. Porosity Data Measured for Calcibon, Standard Deviation Within Brackets

Method	Porosity (%)
Water evaporation	34.7 (0.6)
Helium pycnometry	37.6 (0.3)
XRD	46.7 (0.3)
Tabulated density (HA)	48.1 (0.3)

4. Weigh the dry sample and calculate the volume of the evaporated water using the difference in weight before and after drying.
5. The porosity should be calculated using Eq. (6).

From the results it is apparent that drying acidic CPCs at RT is advantageous over drying at higher temperatures. Although drying of other types of cements normally is performed at higher temperatures^{6,13} to ensure that no water is adhered to the sample surface, the results herein show that acidic CPC cannot be treated in the same way. An elevated drying temperature was found to induce a phase transformation from brushite to monetite, see Figure 2, with the accompanying release of water, see Eq. (7). This transformation has previously been suggested to occur first at 180°C,²⁹ but it was here evident that it occurred earlier. It is not unexpected that brushite could easily be dehydrated into monetite, since monetite is the more stable phase.³⁰ Moreover, it is not surprising that brushite was the main product after setting of acidic CPCs, as the high nucleation energy of monetite, connected to the high energy needed to dehydrate calcium ions in water.³¹ It has previously been found that if these CPCs set with a limited amount of water at 37°C, brushite can be decomposed to monetite and water is released to enable the continuation of the reaction.³² The same decomposition to monetite and release of water was also noted in the present investigation when drying at both 37 and 60°C.



The XRD results showed a small increase in β -TCP content after drying. This is not likely the true scenario, as β -TCP does not contain any crystallization water, and nothing is therefore expected to happen during drying. The rationale for the lower amount of β -TCP before drying is probably the difficulties in XRD sample preparation for wet samples. Aggregation of grains during grinding occurs to a great extent in wet samples, which results in larger particles in wet samples than in dried samples after grinding. Due to the high mass absorption coefficient for β -TCP and the low mass absorption coefficient for brushite compared to β -CPP and monetite, see Table I, there will be an underestimation of the amount of β -TCP and an overestimation of the amount of brushite in a coarsely ground sample; explaining the differences between the wet samples and the RT dried samples. These results furthermore imply that analysis of

dried samples is preferred over analysis of wet samples since the former can be ground more thoroughly.

Previous porosity studies on brushite cements have mostly been performed on samples dried at temperatures higher than RT.^{8,9} It is from hereon recommended not to use higher temperatures for drying of acidic CPCs, as this can induce a phase transformation. Drying at higher temperatures will result in misleading porosity values. A phase transformation to a denser phase (e.g., from brushite to monetite) will result in an increased skeletal density, and at the same time give a lower apparent density since some crystallization water has evaporated. If Eq. (1), which calculates the porosity of such a material, is considered, it is clear that the porosity will be higher for these materials than for a material where the transformation has not occurred.

While both drying in a desiccator at RT and drying in vacuum at RT showed similar results, with mass losses of approximately 17% and cements that could be considered dry after 24 h, drying in vacuum was chosen as the preferred method as it showed a faster initial weight loss and has no restrictions related to saturation of the drying material, and so forth. Furthermore, the vacuum drying technique seemed to give the lowest variation between different porosity methods, which was likely due to a more complete drying of the samples. Seemingly dry desiccator samples can still bear some traces of adhered water, which will affect the analysis, especially helium pycnometry and methanol resaturation.

Methanol exchange showed, similar to drying at elevated temperatures, a phase transformation from monetite to brushite, which explains the high porosity values achieved for this method. Although this analysis was performed at RT, the phase transformation occurred fast. This is likely due to an interaction between the small methanol molecule and brushite. Methanol probably replaces the crystallization water in brushite and is then released with lower energies than what are needed for the release of the crystallization water alone. This is in line with results found for other calcium containing cements, where a strong interaction between methanol and calcium was seen.³³ However, this has to be further investigated for CPCs before final conclusions can be drawn. These results indicate that methanol exchange is not a good method for porosity measurements of acidic CPCs. However, the exchange method could possibly be used with other alcohols with a higher molecular weight, such as iso-propanol, which should not interact to the same extent with the calcium phosphates.³³ However, this also needs further evaluation.

The porosity methods tested gave a diversity of results within each drying method, with varying trends depending on the drying temperature. This can be explained by the fact that all methods assume a constant apparent volume. For samples where a phase transformation occurred (i.e., samples dried at higher temperatures than RT) a decrease in the apparent volume can be assumed. Such a decrease would result in an increase in the apparent density, thus the methods using apparent density according to Eq. (1), XRD

calculations and helium pycnometry would underestimate the porosity if a constant apparent volume was assumed. However, for the other methods (methanol resaturation and water evaporation) according to Eqs. (2) and (6), the porosity would be overestimated when a fixed apparent volume was assumed. This is clearly visualized if the two extremes, drying in desiccator at RT and at 60°C, are compared. Methanol resaturation and water evaporation follows the same trend for the two cases, that is, methanol resaturation gives a lower value than water evaporation, while XRD calculations always gives a higher value than helium pycnometry (however, not significant). The difference lies in comparing the two groups. For the 60°C group, both XRD calculations and helium pycnometry likely give lower results than the true value, while water evaporation and methanol resaturation give too high values; and for this group, water evaporation ($56.5 \pm 0.7\%$) gives a significantly higher value than XRD calculations ($54.9 \pm <0.1\%$), while for the RT group water evaporation ($28.0 \pm 3.6\%$) gives a significantly lower value than helium pycnometry ($32.5 \pm 0.1\%$), see Table III. The rest of the discussion will therefore focus on the samples dried at RT, where no phase transformation has occurred, and where the apparent volume likely stayed the same throughout the drying procedure.

Which method then gives the best estimation of the actual porosity of the material? Water evaporation where drying at RT is used likely gives a slight underestimation of the porosity. Since the cement is very hygroscopic it is likely that some water will be adhered to walls, inside small voids, and so forth, even after drying. This water will be so tightly bound to the cement that it would be almost impossible to remove without increasing the temperature and thus causing phase transformations or decomposition of the different phases. This was especially seen when drying in a desiccator. Vacuum drying seemed to remove adhered water better and therefore give more repeatable results. It can also be debated whether or not all pores are actually filled with water when the mass of the wet sample is measured. However, after storing the samples in PBS in vacuum for 2 min no weight change was seen and it can thus be assumed that all voids were filled with water. Previous studies have also shown that water can penetrate pores as small as 0.5 nm,⁶ and pores smaller than this are likely not relevant for the porosity measurements of these materials.

Porosity measurements using helium pycnometry generally gave higher values than water evaporation for samples dried at RT (both for acidic CPC and Calcibon). Supposedly, this method should give the best estimations of the porosity since it can penetrate the smallest cavities due to the small size of the helium atom. However, it has been shown for other types of ceramics that a collapse of the structure can occur during drying⁵ and result in an increased porosity.⁵

XRD calculations showed the highest porosity for all samples dried at RT (also Calcibon). This is not surprising since XRD only measures the crystalline phases of the sample. The less dense amorphous phases are not measured and XRD calculations generally overestimate the skeletal density, and consequently the porosity of the sample. This

was even more apparent for the Calcibon[®] sample, which is not unexpected since PHA cements normally have smaller grains/are more X-ray amorphous than brushite/monetite cements. This is illustrated in Figure 3, where the peaks from HA are much broader (especially around 32°) than the peaks for all other phases.

Methanol resaturation showed slightly lower or similar porosities to the water evaporation method. This has previously been seen for cements in the C-S-H system⁵ and is explained by the larger size of the alcohol molecule compared to the water molecule. Furthermore, the larger difference between water evaporation porosity and methanol resaturation porosity, for samples dried at elevated temperatures, is likely due to the high fraction of very small pores that are formed during dehydration of the crystallization water, which the larger methanol molecule cannot penetrate.

MIP is the method that gives most information about the sample: from this method not only total porosity, but also the distribution of pore interconnections is given, see Figure 5. However, MIP normally underestimates the porosity since it cannot penetrate all pores due to the low wetting capabilities and large molecular size of mercury.⁶ Furthermore, the high pressure used during analysis can destroy the pore walls and result in unreliable data.

Validation of the accuracy of the water evaporating method using a basic CPC (Calcibon) instead of an acidic CPC gave quite intriguing results. The calculated skeletal density using XRD data was 3.07 g/cm³, whilst the measured density with helium pycnometry was almost 20% lower, 2.63 g/cm³. Furthermore, the calculated skeletal density was in turn slightly lower than the density of pure HA (3.16 g/cm³), which is, as previously mentioned, normally used for porosity calculations of basic CPCs. From XRD data it was also clear that the cement contained only around 40 wt % HA, and using the density of HA for calculating the porosity would hence be quite misleading. This result is similar to results previously presented for other basic CPCs where large amounts of the starting material are still present after final setting.^{14,15} It should, however, be noted that basic CPCs have a longer setting time than acidic CPCs and the porosity is normally evaluated after one week of curing; however, in this study 24 h was chosen in order to have the same conditions for both cement types. Water evaporation gave the lowest porosity (34.7 ± 0.6%), and as previously discussed, helium pycnometry gave slightly higher results (37.6 ± 0.3%), similar to the acidic CPCs dried at RT. Furthermore, the difference between water evaporation and helium pycnometry was much smaller than the difference between helium pycnometry and the XRD calculations (46.7 ± 0.3), which indicates that the porosity should be somewhere around 36% for this material, and not as high as 47%.

The results presented in this study show that acidic CPCs should be dried at low temperatures, not above RT. They also show that drying in vacuum for 24 h is enough to achieve completely dry samples, and that no further drying should be performed due to the phase transformation of brushite to monetite. Furthermore, it was shown that water

evaporation could indeed be used as a reliable estimation of the porosity of CPCs, and in the case of basic CPCs it is likely even better than the already established method of using the theoretical bulk density of HA to calculate the porosity. It can be debated whether or not helium pycnometry gives more accurate results; however, water evaporation has the advantage of being a fast and easy method and, particularly, no elaborate equipment is needed. This also means that more samples can be analyzed, giving better statistical power to the measurements (in this work 20 samples for water evaporation compared to 3 for helium pycnometry) The method could hence preferably be used to compare porosity between samples within a study. It should, however, be mentioned that the term porosity, although easily defined, was shown to not be an absolute term. It is thus hard to compare studies made by different research groups. Furthermore, it should be mentioned that to achieve a complete picture of the true sample porosity, it is of highest importance to measure the porosity in as many ways as possible.

CONCLUSIONS

- Weighing of a sample before and after drying is a fast and easy way to measure porosity without affecting the composition or structure of the material.
- The accuracy of the water evaporation method was seen to be similar to that of helium pycnometry, which is considered one of the best methods to measure porosity. However, with the water evaporation method, more samples can be used and the accuracy of the measurement can be improved without being time consuming. Also, no elaborate equipment is needed.
- Vacuum drying for 24 h at RT is sufficient to completely dry the cement.
- Drying of CPCs that are prone to undergo phase transformation should be performed at low temperatures (RT or lower).
- Porosity calculation using the theoretical density of HA did not give reliable results for the investigated hydroxyapatite cement.

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